B2

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 743 067 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
27.11.2002 Bulletin 2002/48

(51) Int Cl.[7]: **A61K 31/715**, A61K 31/70, C08B 37/08, C08B 11/14, C08B 37/00

(21) Application number: **96303441.8**

(22) Date of filing: **15.05.1996**

(54) Use of urea and thiourea compounds for elimination or detoxofication of superantigens from body fluids

Verwendung von Harnstoff- und Thioharnstoffverbindungen zur Entfernung oder Entgiftung von Superantigenen aus Körperflüssigkeiten

Utilisation de composés d'urée et de thiourée pour l'élimination ou détoxification des superantigènes des fluides corporels

(84) Designated Contracting States:
BE CH DE ES FR GB IT LI NL SE

(30) Priority: **16.05.1995 JP 11717595**
28.12.1995 JP 34420495

(43) Date of publication of application:
20.11.1996 Bulletin 1996/47

(73) Proprietor: **TORAY INDUSTRIES, INC.**
Tokyo 103 (JP)

(72) Inventors:
- **Fukuyama, Mayumi**
  Kusatsu-shi, Shiga 525 (JP)
- **Miwa, Keishi Toray Takatsuki-ryo 515**
  Takatsuki-shi, Osaka 569-11 (JP)
- **Ishikawa, Kazuo**
  Otsu-shi, Shiga 520 (JP)

(74) Representative: **Coleiro, Raymond et al**
MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP (GB)

(56) References cited:
EP-A- 0 355 047
DE-A- 1 644 815
DE-A- 3 438 527
US-A- 4 267 313

- **PATENT ABSTRACTS OF JAPAN vol. 012, no. 181 (C-499), 27 May 1988 & JP 62 288602 A (AGENCY OF IND SCIENCE & TECHNOL), 15 December 1987,**
- **DATABASE WPI Section Ch, Week 8836 Derwent Publications Ltd., London, GB; Class A96, AN 88-254713 XP002062558 & JP 63 186 660 A (UBE IND LTD) , 2 August 1988**
- **MUZZARELLI ET AL.: "Chitin-based poly(urea-urethane)s" J. BIOMATER. SCI. POLYM. ED., vol. 6, no. 6, 1994, pages 541-547, XP002062553**
- **MOORE ET AL.: "Reactions of chitosan: 4. Preparation of organosoluble derivatives of chitosan" INT. J. BIOL. MACROMOL., vol. 4, no. 4, 1982, pages 246-249, XP002062554**
- **AIBA ET AL.: "Covalent immobilization of chitosan derivatives onto polymeric film surfaces with the use of a photosensitive hetero-bifunctional crosslinking reagent" BIOMATERIALS, vol. 8, no. 6, 1987, pages 481-488, XP002062555**
- **DATABASE WPI Section Ch, Week 8823 Derwent Publications Ltd., London, GB; Class A11, AN 88-157861 XP002062559 & JP 63 097 633 A (FUJI SPINNING CO LTD) , 28 April 1988**
- **PATENT ABSTRACTS OF JAPAN vol. 096, no. 004, 30 April 1996 & JP 07 330809 A (NIPPON PAPER IND CO LTD), 19 December 1995,**
- **BUTCHER ET AL.: "Immunologic Studies of Hollow-Fiber Dialyzer Extracts" ARTIFICIAL ORGANS, vol. 8, no. 3, 1984, pages 318-328, XP002062556**
- **FAN'KOVSKAYA ET AL.: "PRODUCTION AND CERTAIN PROPERTIES OF STAPHYLOCOCCAL TOXIN, INDUCING TOXIC SHOCK SYNDROME" MOL. GENET. MIKROBIOL. VIRUSOL., vol. 7, 1987, pages 23-27, XP002062557**

Printed by Jouve, 75001 PARIS (FR)

EP 0 743 067 B1

Description

[0001] The present invention relates to a material for use in the detoxification or elimination of super antigens such as staphylococcal enterotoxin and streptococcal exotoxin. This material binds with super antigens existing in a protein solution at a high concentration such as in the human blood etc., it is preferably used as an (antidotal) medicine for reducing or eliminating toxic activity of super antigens, as a purification column for eliminating the super antigen or as a wound dressing material.

[0002] Super antigens are a group of proteins which can directly bind with major histocompatibility antigen class II proteins (hereinbelow called "MHC class II" in some cases) on an antigen presenting cell without the necessity for passage, during processing, through the antigen presenting cell to be different from that for conventional antigens and furthermore, stimulate a T-cell by forming a complex with this MHC class II and T cell. Several restrictions exist in binding the T-cell for the conventional antigens, and the number of T-cells reacting with this is usually at most one per ten thousand, but as the super antigen binds only to the variable region of the β - chain of the T-cell receptor, certain kinds of super antigens stimulate one T-cell among five T-cells. As the result, it is thought that super antigens stimulate extraordinarily the immune system to generate fevers, rash and hypotension during sepsis and vomiting during food poison or autoimmune diseases (D.L.Murray et al, American Society of Microbiology News, (1995), 61(5), 229). As the super antigens, staphylococcal enterotoxin, Streptococcal exotoxin, Yersinial exotoxin, certain virus proteins and heat shock proteins have been confirmed and it is possible that other super antigens will be found in future.

[0003] Up to this time, as those substances which have affinity with these super antigens, antibodies to these super antigens (P.M.Rosten et al., Journal of Clinical Microbiology, (1987), 25(2), 327), major histocompatibility antigen class II proteins and a part thereof (J.K.Russell et al. Biochemical and Biophysical Research Communications, (1990), 168, 696) and ion exchange resins (H. Igarashi et al., Infection and Immunity, (1984), 44(1), 175) are known and they have been used as binding substances for adsorbing super antigens in blood and culture supernatant. However, most of these binding substances are proteins and peptides and they are easily deactivated by sterilization. In addition, affinity between ion exchange resins and super antigens is easily reduced by the influence of pH of the solution; specificity is decreased in the neutral region. Therefore, they are not suitable as a material with sufficient affinity for super antigens in solutions having a high protein concentration as blood, foods etc., where the pH should be kept neutral.

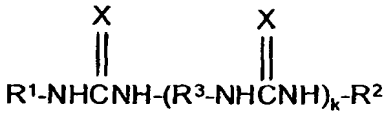
[0004] A body fluid purifying material, for removing organ transplantation rejection factors in blood, and which comprises a water-insoluble compound obtained by reaction between a polymeric compound having hydroxyl groups and a compound having at least one isocyanate group is disclosed in US 5144013.

[0005] The present invention seeks to address the problem of solving the disadvantages of conventional technologies by providing for the use of a material with excellent selective affinity for super antigens even in a high protein concentration solution at a pH in the neutral region, with residual activity after sterilization and which is inexpensive. Namely, the material employed in the present invention has a high affinity for super antigens and it can bind with super antigens existing in body fluids such as blood and urine, foods, drinks and medicines. It is possible by such binding that the toxin-like activities of the super antigens can be eliminated (detoxification) by changing such properties of the super antigens as their conformations or by shielding the binding sites with MHC class II or/and T-cells. Namely, when the material is used as a medicine, it is possible to effectively treat the effects of food poisoning, sepsis and autoimmune diseases or to prevent them from occurring. In addition, if this material is water-insoluble, it becomes possible by using this to eliminate super antigens from body fluids such as blood and urine, foods, drinks and medicines to treat the effects of food poisoning, sepsis and autoimmune diseases and to prevent them from occurring. In particular, it is suitable as a body fluid purifying column for eliminating super antigens or as a super antigen-adsorbing wound dressing material.

[0006] In addition, it may be used as a quantitative measuring material and it is possible to diagnose food poisoning, sepsis and autoimmune diseases. The present invention provides a material which enables diagnosis and therapy of these diseases and prevents them from occurring.

[0007] We found that a material containing a urea bond or a thiourea bond has an affinity with staphylococcal enterotoxin and Streptococcal exotoxin. That is, a first aspect of the present invention provides the use, in the preparation of an agent employed in the treatment of a human or animal body for elimination or detoxification of super antigens, of a material having therein a urea bond or thiourea bond, and which material additionally has therein an aromatic ring and/or a group capable of forming a hydrogen bond. A second aspect provides the use of such a material for in vitro elimination or detoxification of super antigens. The material preferably comprises a group capable of forming a hydrogen bond and an aromatic substituent. Thus, in this aspect of the invention the material may be employed as a medicament, for example, on a wound dressing, or as a sorbing agent during removal of either blood or plasma out of a human or animal body, or during in vitro sorption procedures, which may be carried out on a body fluid such as blood, plasma or serum.

[0008] A third aspect of the present invention provides a urea or thiourea compound, for use in the treatment of a human or animal body for elimination or detoxification of super antigens, which compound has the following formula (I^1):



wherein

X is O or S;
k is 0 or a positive integer; and
each of R^1, R^2 and R^3, which may be the same as or different from one another, is any one of a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent provided that the compound has therein a unit having an amino group as a group capable of forming a hydrogen bond and a unit having an aromatic substituent.

[0009] Where $k \geq 2$, it is preferable that a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent alternate with one another.

[0010] As a group capable of forming a hydrogen bond, an amino group or a hydroxyl group, particularly, a secondary or tertiary amino, group is preferable. More preferably, in such a compound,

(i) R^1 and/or R^3 contains a structure of formula (II)

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II);$$

or

(ii) at least one of R^1, R^2 and R^3 contains a structure of the formula (III)

wherein R^4 is hydrogen or a $C_{1\text{-}10}$ alkyl group and wherein in each of formulae (II) and (III), each of m and n, independently of one another, is selected from 0 and 1-10.

[0011] A fourth aspect of the present invention provides a body fluid purifying column comprising the above mentioned material of formula (I').

[0012] A fifth aspect of the present invention provides a wound dressing material comprising a material having a urea bond or thiourea bond and additionally an aromatic ring and/or a group capable of forming a hydrogen bond.

[0013] Embodiments of the invention will now be described in more detail.

[0014] There are no special limitations as the substituent of the urea bond or the thiourea bond and aliphatic compounds such as a hexyl group, octyl group and dodecyl group and alicyclic compounds such as cyclohexane and cyclopentane may be used, but aromatic compounds such as those containing a phenyl group, naphthyl group and anthranyl group are more preferably used. In addition, derivatives such as those containing an aminohexyl group, monomethylaminohexyl group, dimethylaminohexyl group, aminooctyl group, aminododecyl group and tolyl group, chlorophenyl group, nitrophenyl group, diphenylmethyl group and aminodiphenylmethyl group are also preferably used. Moreover, compounds containing, as substituents, a group capable of forming hydrogen bonds such as an amino group, hydroxyl group, carboxyl group and mercapto group are preferably used. For example, such compounds as those having a hydroxyl group, for example, hydroxypropane, 1,3 diamino-2-hydroxypropane, hydroxybutanone, hydroxybutyric acid and hydroxypyrimidine and glucides such as monosaccharides, oligosaccharides and polysaccharides such as glucose, glucosamine, galactosamine, maltose, cellobiose, sucrose, agarose, cellulose, chitin, chitosan and derivatives thereof, and those compounds having amino group, for example, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, dipropylenetriamine, polyethyleneimine, N-methyl-2,2'-diaminodiethylamine are preferably used. The material of the present invention has most preferably both a group derived from an aromatic

compound and a group capable of forming a hydrogen bond derived from a compound such as an amino group - or a hydroxyl group-containing compound (including glucides or their derivatives) as substituents on a urea bond or a thiourea bond.

[0015] In addition, any of monomers, oligomers or polymers can be employed as a material used in accordance with the present invention. Thus, compounds such as polymers derived wholly or in part from compounds having the above described substituents are also in the range of materials of the present invention. Namely, as polymers which partly or wholly comprise units having the above described substituents, those having a repeating unit of synthetic polymers such as nylon, polymethyl methacrylate, polysulfone, polystyrene, polyethylene, polyvinyl alcohol and polytetrafluoroethylene and natural polymers such as cellulose, collagen, chitin, chitosan and their derivatives, are preferably used. Namely, it is preferable to introduce urea bonds or thiourea bonds into these synthetic polymers prepared by homopolymerization, copolymerization or blending or into natural polymers. In addition, those products prepared by coating an inorganic material such as metals, ceramics and glass with an appropriate polymer are also preferably used.

[0016] In addition, polyurea or polythiourea wherein a plurality of urea bonds or thiourea bonds exist in the molecular structure is preferable as a material for use in the present invention. In this case, any one of the above described substituents can be used as the substituent of the urea bond or the thiourea bond, it is most preferable to incorporate both an aromatic compound and a compound having a group capable of forming a hydrogen bond such as amino group or hydroxyl group-containing compound (including glucides and their derivatives).

[0017] The material of the present invention can be synthesized by generally known methods. For example, when a urea bond or a thiourea bond is introduced into an aliphatic compound and an aromatic compound, a method wherein an isocyanate derivative or an isothiocyanate derivative is reacted with an amino compound can be used. As the isocyanates or the isothiocyanates, for example, aliphatic isocyanates or isothiocyanates such as ethyl isocyanate, stearyl isocyanate, n-butyl isocyanate, iso-butyl isocyanate, n-propyl isocyanate, methyl isothiocyanate, ethyl isothiocyanate, n-butyl isothiocyanate, benzyl isothiocyanate, hexamethylenediisocyanate, cyclohexyl isocyanate, cyclohexyl-isothiocyanate and cyclohexyldiisocyanate can be used, but aromatic isocyanates or isothiocyanates such as phenyl isocyanate, chlorophenyl isocyanate, fluorophenyl isocyanate, bromophenyl isocyanate, nitrophenyl isocyanate, tolylphenyl isocyanate, methoxyphenyl isocyanate, 1-naphthyl isocyanate, 4,4'-diphenylmethanediisocyanate, 3,3',5,5'-tetraethyl-4,4'-diisocyanatediphenylmethane, phenyl isothiocyanate, chlorophenyl isothiocyanate, fluorophenyl isothiocyanate, nitrophenyl isothiocyanate, tolyl isothiocyanate, methoxyphenyl isothiocyanate and 1-naphthyl isothiocyanate, are more preferably used. In addition, as amino group of the amino compounds used in the present invention, any of primary amino group, secondary amino group or tertiary amino group can be used and as amino compound, for example, any one of sec-octyl-amine, 6-amino-n-caproic acid, 3-amino-1-propene, α-amino-isobutyric acid, aminopyridine, aminobenzenesulfonic acid, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, dipropylenetriamine, N-methyldiaminodiethylamine, polyethyleneimine etc., can be used; however taking the reactivity of the amino group into consideration, it is preferable to have a primary amino group at at least one reaction site. In addition, amino compounds with a hydroxyl group can be more preferably used. Namely, aliphatic amines such as 2-ethanolamine, 3-propanolamine, 6-hexanolamine, 1,3-diamino-2-hydroxypropane and glucamine and derivatives of N-methyl-1,3-diaminopropanol or aromatic amines such as 4-aminophenol, diaminophenol, aminohydroxypyrimidine, diaminohydroxypyrimidine and diaminohydroxypyrazole or amino acids such as serine and tyrosine can be used. In addition, it is preferred that an amino compound with a hydroxyl group is synthesized from a compound only with a hydroxyl group or a compound only with an amino group by reacting it with epichlorohydrin and an amino compound or 1,3-dibromo-2-hydroxypropane etc. In this case, the mixing ratio of the amino compound to an isocyanate derivative or an isothiocyanate derivative can be arbitrarily selected and it is preferable that the amount of amino group is an equimolecular quantity or excess to the amount of isocyanate group to suppress the reaction of the hydroxyl group with isocyanate or isothiocyanate group. In addition, when urea bonds or thiourea bonds are introduced into a glucide, the same method as described above can be used. Namely, when a glucide with an amino group such as chitosan or glucosamine is used, the above described isocyanate derivative or isothiocyanate derivative can be reacted. In the case of a glucide that has no amino group such as cellulose, after the hydroxyl group of the glucide is activated using epichlorohydrin or trisilylchloride, an amino group is introduced by reacting it with ammonia or diaminoethane and urea bonds or thiourea bonds can be introduced into a glucide utilizing this amino group.

[0018] In addition, when the material of the present invention is an oligomer or a polymer, for example, a method wherein an oligomer or a polymer with an isocyanate group, a carboxyl group or an active ester group of a carboxylic acid such as succinimide group is reacted with the amino group of a urea derivative or a thiourea derivative, is preferably used. As the amino group used in the reaction, because of the low reactivity of the terminal amino group of the urea bond or the thiourea bond, it is preferable to use an amino group existing on another position. In addition, a method wherein an oligomer and a polymer each with an amino group or an oligomer and a polymer wherein an amino group is introduced by using ammonia, diaminoethane, 1,3-diaminopropane or 1,3-diamino-2-hydroxypropane are reacted with an isocyanate derivative or an isothiocyanate derivative, is preferable. Functional groups such as amino groups, isocyanate groups, carboxyl groups or an active ester group of a carboxylic acid such as a succinimide group can be

introduced if necessary, into an oligomer and a polymer.

[0019] In addition, when the material of the present invention is polyurea or polythiourea, for example, a method wherein a polyisocyanate derivative or a polyisothiocyanate derivative is reacted with a polyamino compound can be used. Ordinarily, as the amount of the reagent, 0.1-5 mole polyamine is preferably used to 1 mole polyisocyanate or polyisothiocyanate. As the polyisocyanate or polyisothiocyanate, hexamethylenediisocyanate, cyclohexyldiisocyanate, tolylene diisocyanate, 4,4'-diphenylmethanediisocyanate, 3,3',5,5'tetraethyl-4,4'-diisocyanate-diphenylmethane, xylylene diisocyanate, methylene-bis(4-phenyl isothiocyanate) etc., are preferably used. In addition, as the polyamino compound, diaminoethane, diaminopropane, 1,3-diamino-2-hydroxypropane, N-methyl-1,3-diamino-2-propanol, diamino-phenol, N,N'-diaminopiperazine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polyethyleneimine, dipropylenetriamine, N-methyldiaminoethylamine etc., are preferably used.

[0020] All the above described reactions are performed as the standard at a reaction temperature of 0-150°C and for a reaction time of 0.1-24 hours. In addition, even though the reaction solvent is not always necessary, but the reaction is ordinarily performed in the presence of a solvent. As the solvents which can be used, aliphatic hydrocarbons such as methanol, ethanol, isopropyl alcohol, n-butanol, hexane, acetone, N,N-dimethylformamide and dimethyl sulfoxide, aromatic hydrocarbons such as benzene, toluene and xylene, halogenated hydrocarbons such as dichloromethane, chloroform and chlorobenzene, ethers such as diethyl ether, tetrahydrofuran and dioxane are cited. The product can be purified by column chromatography and recrystallization after the reaction liquid is treated by such an ordinary aftertreatment as filtration and concentration. In addition, in the case of a water-insoluble material, washing it by using a glass filter is also a preferable method.

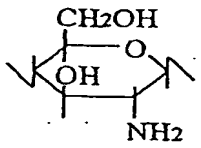
[0021] In the present invention water-insoluble materials are preferably used, for example, for a super antigen elimination column, a wound dressing material or a quantitative measuring material. There is no special limitation on their shapes and when they are used as an elimination column, shapes such as beads, fibers, hollow fibers, yarns, nets, braids, woven or knitted fabrics of coarse structure (randomly packed, spirally wound or packed with fragments thereof) are preferable and when they are used for a quantitative measuring material, shapes such as beads, plates, yarns, nets, braids, woven or knitted fabrics of coarse structure (randomly packed, spirally wound or packed with fragments thereof) are preferable and in the case of wound dressing materials, shapes such as fabrics and films are preferable. As materials having urea bonds, porous chitosan beads, "CHITOPEARL BCW-3001" and "CHITOPEARL BCW-3501" (Fuji Spinning Co., Ltd) are commercially available. However, these chitosan beads have been used as carriers for enzyme immobilization and are not yet known to have an affinity for super antigens. Chitosan, crosslinked with organic diisocyanate, is also proposed in JP-A-63097633. Also polyether urethane urea has urea bond and has been used as a material for medical use; however, it does not have affinity for super antigens.

[0022] Especially preferred embodiments will now be described in more detail with reference to the accompanying drawings and the following Examples. In the drawings,

Figure 1 shows an infrared spectrum of chitosan beads modified with p-chlorophenyl isocyanate;
Figure 2 shows the results of adsorption tests on super antigens by means of circulation method; and
Figure 3 shows an infrared spectrum of a polyurea derivative.

Example 1 Introduction of urea bonds into chitosan beads and a super antigen adsorption test using the beads

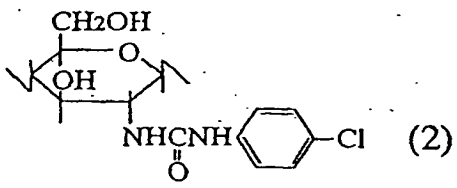
[0023] Beads of chitosan ("CHITOPEARL AL-01" manufactured by Fuji Spinning Co., Ltd.) with a structural formula (1), having a sedimentary volume of 12 ml, a dry weight of 1.0g and a particle diameter of 0.1 mm were stirred in 20 ml N,N-dimethyl-formamide for five minutes. Then the beads and the solution were separated by means of a glass filter.



(1)

[0024] This operation which needed 5 minutes per operation was repeated 20 times to substitute N,N-dimethylformamide for the water content. These beads were gradually added into 100 ml N,N-dimethylformamide in which 1 g p-chlorophenyl isocyanate was dissolved and the mixture was reacted for 1 hour at room temperature while it was stirred. Thereafter, the beads and the solution were separated using a glass filter and washing was performed by stirring these beads in 20 ml N,N-dimethylformamide for 5 minutes. This washing operation was repeated 20 times to eliminate completely unreacted p-chlorophenyl isocyanate. Then, a washing operation with distilled water was performed in the

same way to substitute distilled water for N,N-dimethylformamide and chitosan beads with a structural formula (2) were obtained. An infrared spectrum of the modified chitosan beads is shown in Figure 1.



(2)

[0025] By using these modified chitosan beads (2) and unmodified chitosan beads (1) as a control, adsorption of four kinds of super antigen, namely, staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C (SEC) and toxic shock syndrome toxin-1 (TSST-1) from a rabbit plasma were performed. The initial concentrations of these super antigens were 1 ng/ml and 1 ml of the above described chitosan beads after being autoclaved under high pressure at 120°C for 20 minutes was added into 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. The concentrations of four kinds of super antigen in the rabbit plasmas after reaction for 60 minutes were measured by means of an enzyme immune assay and the results were shown in Table 1. As shown by this result, super antigen adsorbability was provided to the chitosan beads by introducing urea bonds.

Table 1

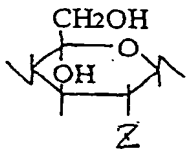
Super antigen adsorption tests for four kinds of super antigen in rabbit plasma samples using modified chitosan beads				
	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
Modified chitosan	513	393	453	288
Unmodified chitosan	1163	1120	960	939

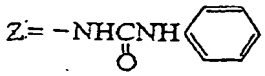
Example 2 A super antigen adsorption test using a modified chitosan bead-circulation method

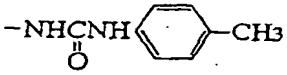
[0026] An adsorption test by means of a circulation method for a super antigen was performed using the unmodified chitosan beads (1) and the modified chitosan beads (2) of Example 1. 1 ml of the above described beads was introduced into a column and 10 ml rabbit plasma in which 1 ng/ml super antigen (TSST-1) was incorporated were circulated at 37°C for 60 minutes. The concentrations in the rabbit plasma after 5, 15, 30, 45 and 60 minutes were measured by means of an enzyme immune assay and the results were shown in Figure 2. Super antigen adsorbability under flow conditions similar to extracorporeal circulation were provided to the chitosan beads by introducing urea bonds in this manner.

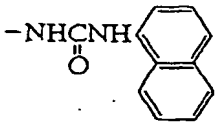
Example 3 Super antigen adsorption tests using seven kinds of chitosan bead wherein urea bonds or thiourea bond were introduced

[0027] Phenyl isocyanate, p-tolyl isocyanate, 1-naphthyl isocyanate, phenyl isothiocyanate and p-chlorophenyl isothiocyanate were respectively reacted with chitosan beads by the same method as in Example 1. In addition, 4,4'-diphenylmethanediisocyanate and hexamethylenediisocyanate were reacted with chitosan beads by the same method as in Example 1 and then, terminal isocyanate groups were hydrolyzed by reacting them with distilled water for 12 hours at room temperature. Thereafter, the beads were washed thoroughly with distilled water. Modified chitosan beads each with a respective structural formula (3)-(9) were obtained by the above described method. Formulae (8) and (9) correspond to "CHITOPEARL BCW-3501" and "CHITOPEARL BCW-3501", respectively.

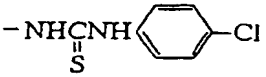


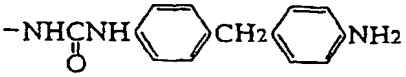
Z=  (3)

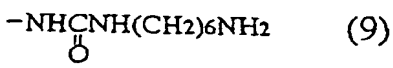
 (4)

 (5)

 (6)

 (7)

 (8)

 (9)

[0028] By using these seven kinds of modified chitosan bead and unmodified chitosan beads as a control, adsorption of super antigen (TSST-1) from rabbit plasma was performed in the same way as in Example 1. The initial concentration of TSST-1 was 1 ng/ml and 1 ml of the above described chitosan beads was added into 10 ml plasma, and the mixture was shaken at 37°C for 60 minutes and the concentrations of TSST-1 in the rabbit plasma samples after reaction were measured by means of an enzyme immune assay. The concentrations of TSST-1 after 60 minutes are shown in Table 2.

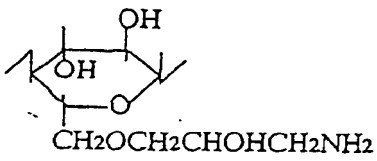
Table 2

Adsorption tests of TSST-1 from samples of rabbit plasma using seven kinds of modified chitosan bead	
Structural formula	TSST-1 concentration (pg/ml)
1	923
3	335
4	415
5	343
6	632
7	290
8	292
9	807

[0029] The structural formula (1) was for unmodified chitosan beads. As these results showed, super antigen adsorbability was provided to the chitosan beads by introducing urea bonds or thiourea bonds.

Example 4 Introduction of urea bonds into cellulose beads and a super antigen adsorption test using the beads

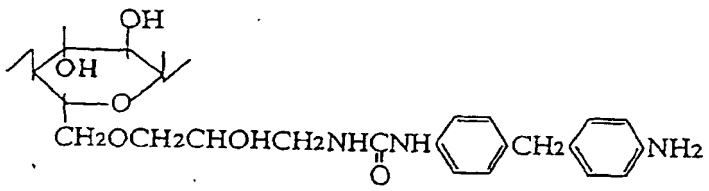
[0030] Aminated cellulose beads ("Amino-Cellulofine" manufactured by Chisso Co., Ltd., Tokyo Japan) with a structural formula (10), having a sedimentary volume of 12 ml and a particle diameter of about 0.2 mm were stirred in 20 ml N,N-dimethylformamide for five minutes. Then the beads and the solution were separated by means of a glass filter.



(10)

[0031] This operation was repeated 20 times to substitute completely N,N-dimethylformamide for water content.

[0032] These beads were gradually added into 100 ml N,N-dimethyl-formamide in which 0.1 g 4,4'-diphenylmethanediisocyanate was dissolved and the mixture was reacted for 1 hour at room temperature while it was stirred. Thereafter, the beads and the solution were separated using a glass filter and washing was performed by stirring these beads in 20 ml N,N-dimethylformamide for 5 minutes. This washing operation was repeated 20 times to eliminate completely unreacted 4,4'-diphenylmethanediisocyanate. Then, it was reacted with the distilled water at room temperature for 12 hours and the terminal isocyanate groups were hydrolyzed to prepare amino groups. Thereafter, by washing thoroughly the beads with distilled water cellulose beads were obtained with a structural formula (11).



(11)

[0033] By using these modified cellulose beads (11) and unmodified cellulose beads (10) as a control, adsorption of a super antigen (TSST-1) from rabbit plasma was performed in the same way as in Example 1. The initial concentration of TSST-1 was 1 ng/ml and 1 ml of the above described cellulose beads was added into 10 ml plasma and the mixture was shaked at 37°C for 60 minutes. The concentration of TSST-1 after the reaction was measured by an enzyme immune assay and the concentration of TSST-1 after 60 minutes are shown in Table 3.

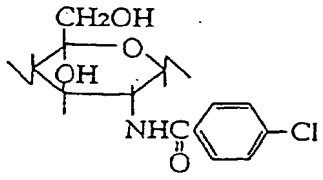
Table 3

TSST-1 adsorption from samples of rabbit plasma using modified cellulose beads	
	Concentration of TSST-1 (pg/ml)
Modified cellulose	485
Unmodified cellulose	946

[0034] As shown by this result, super antigen adsorbability was provided to the cellulose beads by introducing urea bonds.

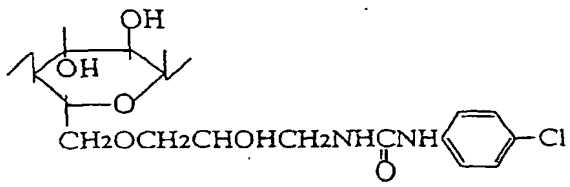
Example 5 (Comparative Example 1) Comparative tests on adsorption functions of TSST-1 between beads with amide bonds and urethane bonds and beads with urea bonds.

[0035] Chitosan beads with amide bonds (structural formula (12)) were prepared by reacting chitosan beads ("CHITOPEARL AL-1" with a structural formula (1)) with p-chlorobenzoyl chloride. This was a product wherein urea bonds of chitosan beads with the structural formula (2) prepared in Example 1 were replaced with amide bonds.

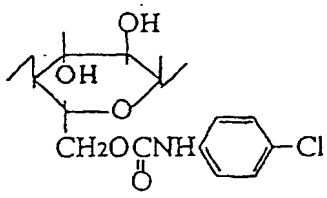


(12)

[0036] In addition, by the same method as that of Example 1, cellulose beads with urea bonds of structural formula (13) were prepared by reacting the aminated cellulose beads ("Amino-cellulofine") used in Example 4 with p-chlorophenyl isocyanate. On the other hand, cellulose beads wherein urethane bonds were introduced (structural formula (14)) were prepared by reacting cellulose beads ("Cellulofine GCL2000") with p-chlorophenyl isocyanate in the presence of triethylamine for 12 hours.



(13)



(14)

[0037] By using these beads, adsorption of super antigen (TSST-1) from rabbit plasma was performed. The initial concentration of TSST-1 was 1 ng/ml and 1 ml of the above described beads was added into 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. The concentrations of TSST-1 in the samples of rabbit plasma after reaction were measured by an enzyme immune assay and the results after 60 minutes are shown in Table 4.

Table 4

Comparative tests on adsorption functions of TSST-1 between beads with amide bonds or urethane bonds and beads with urea bonds (in rabbit plasma)		
Structural formula	Bonding mode	Concentration of TSST-1 (pg/ml)
(2)	Urea bond	310
(12)	Amide bond	947
(13)	Urea bond	398
(14)	Urethane bond	972

[0038] As shown by this result, super antigen adsorbability was not provided by introducing amide bonds and urethane bonds and only in the case of urea bonds was super antigen adsorbability provided.

Example 6 Confirmation of super antigen specificity

[0039] By using modified chitosan beads (the structural formula (2)) prepared in Example 1, adsorbability to TSST-1 as a super antigen was investigated and adsorbabilities to bovine serum albumin (BSA) and human immunoglobulin G (IgG) as non-super antigens were investigated. Each protein was dissolved in rabbit plasma so as to obtain the concentration of 1 ng/ml. 1 ml of the above described beads was added into 10 ml of the plasma, the mixture was shaken at 37°C for 60 minutes and the concentration of the protein in the plasma after reaction was measured by the enzyme immuno assay. The concentrations of the protein after 60 minutes are shown in Table 5. As these results showed, super antigen adsorbability was provided by introducing urea bonds but there was no adsorbability to other proteins while high specificity to super antigens was exhibited.

Table 5

Adsorption characteristics of various proteins using modified chitosan beads	
	Concentration of protein
TSST-1	345 pg/ml
Bovine serum albumin	946 pg/ml
Immunoglobulin G	946 pg/ml

Example 7 Preparation of polyurea derivatives

[0040] 0.32 g 1,3-diamino-2-hydroxypropane (hereinafter abbreviated as DAHP) was dissolved in 40 ml dimethyl sulfoxide (hereinafter abbreviated as DMSO). 10 ml DMSO solution in which 0.63 g 4,4'-diphenylmethanediisocyanate (hereinafter abbreviated as MDI) was dissolved were dropped into this solution while it was stirred. After the whole amount of 10 ml had been added, reaction was performed at 25°C for one hour. Thereafter, 50 ml distilled water were added into the reaction liquid while it was stirred. A white precipitate which formed here was recovered by centrifugal separation and the recovered precipitate was washed five times with 50 ml methanol. Then, the precipitate was dried under vacuum to obtain 0.88 g polyurea derivative (hereunder abbreviated as DAHP polyurea). An infrared spectrum of the polyurea derivative is shown in Figure 3. As shown in Figure 3, the existence of hydroxyl group and urea bond were confirmed. Similarly, another polyurea derivative (hereunder abbreviated as DAP poyurea) was obtained using 1,3-diaminopropane (hereunder abbreviated as DAP) instead of DAHP.

Example 8

[0041] Adsorption tests of super antigens using polyurea derivatives prepared in Example 7 were performed by the same method as that in Example 1. For use as a control, a polyurethane derivative was prepared in the same way as Example 7 except that 1,3-propanediol was used instead of DAHP, triethylamine was included in the reaction mixture and the reaction was performed for 12 hours.

[0042] The initial concentrations of SEA, SEB, SEC and TSST-1 were 1 ng/ml and each 1 ml of DAHP polyurea, DAP polyurea, polyurethane was added in 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. All of DAHP polyurea, DAP polyurea and polyurethane were used after high pressure steam sterilization at 121°C for 20 minutes. The concentrations of four kinds of super antigen in rabbit plasma after 60 minutes reaction were measured

by enzyme immuno assay and the results are shown in Table 6. As these results showed, although polyurethane does not adsorb super antigens, it became clear that polyurea adsorbs super antigens and by introducing hydroxy groups to the polyurea, the adsorbability was improved.

Table 6

Adsorption tests of four kinds of super antigen in rabbit plasma using polyurea				
	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
DAHP polyurea	320	357	333	435
DAP polyurea	728	810	735	749
polyurethane	925	880	956	890

Example 9 Preparation of polystyrene fiber with a hydroxyl group-containing urea derivative on its side chain

[0043] Islands-in-a-sea type composite fiber described in U.S. Patent No. 4,661,260 (thickness: 2.6 denier; number of the islands:16) comprising of 50 wt parts of sea component (mixture of 46 wt parts of polystyrene and 4 wt parts of polypropylene) and 50 wt parts of islands component (polypropylene) were reacted in a mixed solution of 50g of N-methylol-α -chloracetamide, 400g of nitrobenzene, 400g of 98% sulfuric acid and 0.85g of paraformaldehyde at 20°C for one hour. Then the fiber was washed with nitrobenzene, and thrown into water to stop the reaction. After that, the fiber was washed again with warm water. Thus, chloroacetoamidemethylated crosslinked polystyrene fiber (hereinafter abbreviated as AMPSt fiber) was obtained.

[0044] 10 g DAHP were dissolved in 500 ml DMSO. 20 g AMPSt fiber (corresponding to 20 mmol chloro content) were added into this solution while it was stirred. The reaction was performed at 25°C for 6 hours. Thereafter, AMPSt fiber was washed on a glass filter with 500 ml DMSO and then, successively with 50 ml N,N-dimethylformamide. After washing, 1 g each of AMPSt fiber was added into 50 ml DMF wherein one of the below described isocyanates or isothiocyanates was dissolved.

Table 7

Isocyanates or isothiocyanates used for reaction with polystyrene fiber	
Reaction product	Isocyanates or isothiocyanates used for reaction
(a)	0.23 g phenyl isocyanate
(b)	0.30 g para-chlorophenyl isocyanate
(c)	0.30 g meta-chlorophenyl isocyanate
(d)	0.30 g ortho-chlorophenyl isocyanate
(e)	0.27 g para-fluorophenyl isocyanate
(f)	0.32 g para-methoxyphenyl isocyanate
(g)	0.26 g para-tolyl isocyanate
(h)	0.32 g para-nitrophenyl isocyanate
(i)	0.33 g 1-naphthyl isocyanate
(j)	0.48 g 4,4'-diphenylmethanediisocyanate
(k)	0.70 g 3,3',5,5'-tetraethyl-4,4'-diisocyanate-diphenylmethane
(l)	0.24 g cyclohexyl isocyanate
(m)	0.33 g hexamethylenediisocyanate
(n)	0.19 g n-butyl isocyanate
(o)	0.26 g phenyl isothiocyanate
(p)	0.33 g para-chlorophenyl isothiocyanate
(q)	0.27 g cyclohexyl isothiocyanate
(r)	0.22 g n-butyl isothiocyanate

[0045] The reaction was performed at 25°C for one hour. Thereafter, the reaction product was washed on a glass filter with 200 ml DMSO and 500 ml distilled water. The compounds obtained from the reaction with each isocyanate or isothiocyanate were referred to respectively as (a)-(r).

[0046] In addition, a sample of AMPSt fiber wherein a p-nitrophenyl group was introduced by reacting p-nitrophenyl isocyanate was added into 100 ml aqueous sodium hydrosulfite solution (0.1 g/ml) and it was converted into a p-

aminophenyl group by reduction at 60°C for 4 hours; it was referred to as the compound (s).

Example 10 Adsorption of super antigens using polystyrene fibers with urea derivatives on their side chains

[0047] Adsorption tests of super antigens using modified polystyrene fibers prepared in Example 9 were performed by the same method as that in Example 1. The initial concentrations of SEA, SEB, SEC and TSST-1 were 1 ng/ml and each 1 g of the modified AMPSt fiber was added in 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. The modified AMPSt fibers were used after high pressure steam sterilization at 120°C for 20 minutes. The concentrations of four kinds of super antigen in rabbit plasma after 60 minutes reaction were measured by enzyme immuno assay and the results are shown in Table 8.

[0048] As a control, an AMPSt fiber (t) wherein amide bonds were introduced instead of urea bonds by reacting benzoyl chloride instead of isocyanate under the same condition was used. In addition to that, an AMPSt fiber (u) wherein phenyl isocyanate was reacted after DAP was reacted instead of DAHP was also evaluated.

[0049] As these results showed, it became clear that as the polystyrene fiber (t) where there was no urea bond exhibited no adsorbability of super antigens; adsorbability of super antigens was exhibited by introducing urea bonds. In addition, it was shown that higher adsorbability of super antigens was exhibited by modifying with an aromatic isocyanate than with an aliphatic isocyanate. In addition, the adsorbability of super antigens was reinforced by introducing hydroxyl groups.

Table 8

Adsorption of super antigens using polystyrene fibers each with a urea derivative on their side chains

Modified AMPSt	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
(a)	450	382	360	462
(b)	330	382	365	399
(c)	332	381	362	352
(d)	450	462	475	488
(e)	400	412	386	428
(f)	723	733	584	669
(g)	448	285	363	433
(h)	621	588	589	603
(i)	425	335	385	418
(j)	352	350	330	320
(k)	768	812	750	796
(l)	766	801	789	732
(m)	682	762	787	698
(n)	702	785	788	776
(o)	463	355	354	477
(p)	336	375	358	401
(q)	770	798	774	762
(r)	777	774	793	802
(s)	822	852	885	856
(t)	975	985	1022	1005
(u)	802	798	822	785

Example 11 Preparation of polystyrene fiber with an amino group-containing urea derivative on its side chain

[0050] 0.8 g triethylenetetramine was dissolved in 500 ml DMSO. 1.0 g AMPSt fiber (which corresponded to 2 mmol chloro content) were added into this solution while it was stirred. The reaction was performed at 25°C for 12 hours. Thereafter, AMPSt fiber was washed on a glass filter with 500 ml DMSO and then, successively with 50 ml N,N-dimethylformamide. After washing, the AMPSt fiber was added into 50 ml DMF in which 0.30 g p-chlorophenyl isocyanate had been dissolved. Thereafter, AMPSt fiber was washed on a glass filter with 200 ml DMSO and then, successively with 200 ml distilled water and AMPSt fiber (v) was obtained.

Example 12 Adsorption of super antigens using polystyrene fibers with an amino group-containing urea derivatives on their side chains

[0051] Using the AMPSt fiber (v) obtained by Example 11, adsorption of super antigens were performed in the same way as in Example 1. As a control, AMPSt fiber (t) used in Example 10 was used. The initial concentrations of SEA, SEB, SEC and TSST-1 were 1 ng/ml and 1 g of the above described AMPSt (v) fiber were incorporated into 10 ml plasma and the mixture was shaken at 37°C for 60 minutes. Each AMPSt (v) fiber was used after being sterilized under high pressure steam at 121°C for 20 minutes. The concentrations of four kinds of super antigen in the rabbit plasmas after reaction for 60 minutes were measured by an enzyme immune assay and the results are shown in Table 9. As shown by this result, it is clear that an amino group-containing urea derivative has super antigen adsorbability.

Table 9

Adsorption of super antigens using polystyrene fibers with an amino group-containing urea derivative on their side chains				
Modified AMPSt	SEA pg/ml	SEB pg/ml	SEC pg/ml	TSST-1 pg/ml
(V)	241	287	302	197
(T)	956	1001	981	975

[0052] As illustrated above, material containing urea bonds or thiourea bonds with excellent selective binding characteristics with super antigens even in a high protein concentration solution in the neutral region and remaining activity even after sterilization and being inexpensive is provided by embodiments of the present invention. In accordance with the present invention, it is possible that the activities of super antigens existing in body fluids such as blood and urine, foods, drinks and medicines can be removed (detoxified), it is possible to treat food poisoning, sepsis and autoimmune diseases and to prevent them from occurring. In addition, as it is possible using water-insoluble materials to eliminate efficiently super antigens from body fluids such as blood and urine, foods, drinks and medicines, by a super antigen eliminating column and a wound dressing material, it is possible to treat food poisoning, sepsis and autoimmune diseases and to prevent then from occurring. In addition, as the material employed in the present invention can be used as a measuring material, it is possible to diagnose food poisoning, sepsis and autoimmune diseases.

Claims

1. Use, in the preparation of an agent employed in the treatment of a human or animal body for elimination or detoxification of super antigens, of a material having therein a urea bond or thiourea bond, and which material additionally has therein an aromatic ring and/or a group capable of forming a hydrogen bond.

2. Use according to claim 1, which material has therein both an aromatic ring and a group which is capable of forming a hydrogen bond.

3. Use according to claim 2, which material is a compound of formula (I)

$$R^1-NHCNH-(R^3-NHCNH)_k-R^2 \qquad (I)$$

which said compound has therein a group capable of forming a hydrogen bond and an aromatic substituent and,

X is O or S;
k is 0 or a positive integer; and
each of R^1, R^2 and R^3 is any one of a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent.

4. Use according to claim 3, wherein, in the material, a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent are alternately repeated.

5. Use according to any preceding claim wherein a group which is capable of forming a hydrogen bond is an amino group.

6. Use according to claim 5, wherein the amino group is a secondary or tertiary amino group.

7. Use according to any one of claims 1 to 4, wherein a group which is capable of forming a hydrogen bond is a hydroxyl group.

8. Use according to claim 7, wherein the hydroxyl group is a hydroxyl group of a glucide.

9. Use according to claim 8, wherein the glucide is at least one of chitosan, cellulose and derivatives of chitosan and cellulose.

10. Use according to any one of claims 1 to 4 wherein, in the material, the aromatic substituent is a phenyl group, a naphthyl group or a derivative thereof, at least one of the hydrogen atoms of which is substituted by F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 or CH_2PhNH_2.

11. Use according to claim 3 or 4, wherein, in the material, R^1 and/or R^3 contain(s)a structure of formula (II)

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II)$$

where n and m are each independently selected from 0 and 1-10.

12. Use according to claim 3 or 4 wherein, in the material, at least one of R^1, R^2 and R^3 contains a structure of formula (III)

$$-(CH_2)_n\underset{\displaystyle R^4}{\underset{|}{N}}(CH_2)_m- \qquad (III)$$

where R^4 is hydrogen or a $C_{1\text{-}10}$ alkyl group and n and m are each independently selected from 0 and 1-10.

13. Use according to claim 3 or 4, wherein, in the material, k is 1 to 200.

14. Use according to any preceding claim, of a material which additionally includes a substrate.

15. Use according to claim 14, wherein the substrate comprises at least one of polystyrene, polysulfone, polymethyl methacrylate and derivatives of any of these.

16. Use according to claim 14 or 15, wherein the substrate is a fiber.

17. Use according to claim 16, wherein the fiber is an islands-in-a-sea type fiber.

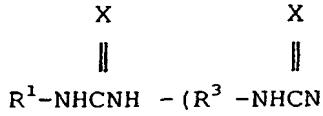
18. Use according to any preceding claim, wherein the material is water-insoluble.

19. Use, for in vitro elimination or detoxification of super antigens, of a material as defined in any one of claims 1 to 18.

20. Use, according to any one of claims 1 to 18, wherein the material is provided in a body fluid purifying column.

21. A wound dressing material comprising a material as defined in any one of claims 1 to 18.

22. A material for use in the treatment of a human or animal body for elimination or detoxification of super antigens, **characterised in that** the material is a compound of formula (I')



$$R^1\text{-NHCNH}-(R^3-\text{NHCNH})_k-R^2 \qquad (I')$$

wherein

X is O or S;
k is 0 or a positive integer; and
each of R^1, R^2 and R^3, which may be the same or different, is any one of a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent provided that the compound has therein a unit having an amino group as a group capable of forming a hydrogen bond and a unit having an aromatic substituent.

23. A material according to claim 22, wherein the amino group is a secondary or tertiary amino group.

24. A material according to claim 22, which also contains a hydroxyl group as a group capable of forming a hydrogen bond.

25. A material according to claim 24, wherein the hydroxyl group is a hydroxyl group of a glucide or a derivative thereof.

26. A material according to claim 22, in which a unit having a group capable of forming a hydrogen bond and a unit having an aromatic substituent are alternately repeated.

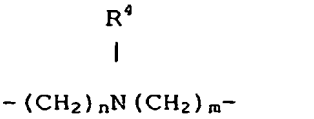
27. A material according to any one of claims 22 to 26, wherein the aromatic substituent is a phenyl group, a naphthyl group or a derivative thereof, at least one of the hydrogen atoms of which is substituted by F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 or CH_2PhNH_2.

28. A material according to any one of claims 22 to 27, wherein R^1 and/or R^3 contain(s)a structure of formula (II)

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II)$$

where n and m are each independently selected from 0 and 1-10.

29. A material according to any one of claims 22 to 27, wherein at least one of R^1, R^2 and R^3 contains a structure of formula (III)



$$-(CH_2)_nN(CH_2)_m- \qquad (III)$$

where R^4 is hydrogen or a $C_{1\text{-}10}$ alkyl group and n and m are each independently selected from 0 and 1-10.

30. A material according to any one of claims 22 to 29, wherein k is 1 to 200.

31. A material according to claim 22 which has a repeating unit of polymethylmethacrylate, polysulfone, polystyrene, or a derivative of any one of these.

32. A body fluid purifying column comprising a material as defined in any one of claims 22 to 31.

33. An in vitro method of removing a super antigen from a fluid by passing the super antigen-containing fluid through a column filled with a material for elimination or detoxification of the super antigen, **characterised in that** the material is as defined in any one of claims 1 to 18.

34. A method according to claim 33, wherein the fluid is blood, plasma or serum.

35. An in vitro method of removing a super antigen from blood or plasma, comprising the successive steps of:

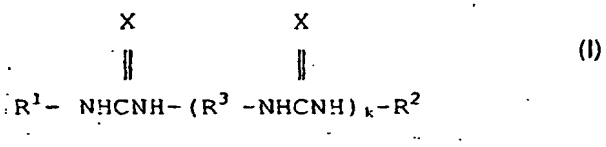
(a) contacting the blood or plasma with a material capable of elimination or detoxification of the super antigen, which material is as defined in any one of claims 1 to 18; and
(b) separating the blood or plasma from the material.

Patentansprüche

1. Verwendung eines Materials, das eine Harnstoffbindung oder eine Thioharnstoffbindung aufweist, zur Herstellung eines Mittels zum Einsatz bei der Behandlung eines Menschen- oder Tierkörpers zur Beseitigung der Entgiftung von Superantigenen, wobei das Material zusätzlich einen aromatischen Ring und/oder eine zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe darin aufweist.

2. Verwendung nach Anspruch 1, wobei im Material sowohl ein aromatischer Ring als auch eine zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe enthalten sind.

3. Verwendung nach Anspruch 2, wobei das Material eine Verbindung der Formel (I):



(I)

ist, wobei in der Verbindung eine zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe und ein aromatischer Substituent enthalten sind, und

X = O oder S ist;
k = 0 oder eine positive ganze Zahl ist; und
R^1, R^2 und R^3 jeweils eine Einheit mit einer zur Bildung einer Wasserstoffbrückenbindung fähigen Gruppe oder eine Einheit mit einem aromatischen Substituenten sind.

4. Verwendung nach Anspruch 3, worin im Material Einheiten mit einer zur Bildung einer Wasserstoffbrückenbindung fähigen Gruppe und Einheiten mit einem aromatischen Substituenten alternieren.

5. Verwendung nach einem der vorangegangenen Ansprüche, worin eine zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe eine Aminogruppe ist.

6. Verwendung nach Anspruch 5, worin die Aminogruppe eine sekundäre oder tertiäre Aminogruppe ist.

7. Verwendung nach einem der Ansprüche 1 bis 4, worin eine zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe eine Hydroxylgruppe ist.

8. Verwendung nach Anspruch 7, worin die Hydroxylgruppe eine Hydroxylgruppe eines Glucids ist.

9. Verwendung nach Anspruch 8, worin das Glucid zumindest eines aus Chitosan, Cellulose und Derivaten von Chitosan und Cellulose ist.

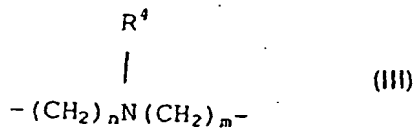
10. Verwendung nach einem der Ansprüche 1 bis 4, worin der aromatische Substituent im Material eine Phenylgruppe, eine Naphthylgruppe oder ein Derivat davon ist, worin zumindest eines der Wasserstoffatome durch F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 oder CH_2PhNH_2 substituiert ist.

11. Verwendung nach Anspruch 3 oder 4, worin R^1 und/oder R^3 im Material eine Struktur der Formel (II):

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II)$$

enthält bzw. enthalten, worin n und m jeweils unabhängig voneinander aus 0 und 1-10 ausgewählt sind.

12. Verwendung nach Anspruch 3 oder 4, worin zumindest einer von R^1, R^2 und R^3 im Material eine Struktur der Formel (III):



(III)

enthält, worin R^4 Wasserstoff oder eine C_{1-10}-Alkylgruppe ist und n und m jeweils unabhängig voneinander aus 0 und 1-10 ausgewählt sind.

13. Verwendung nach Anspruch 3 oder 4, worin im Material k = 1 bis 200 ist.

14. Verwendung eines Materials nach einem der vorangegangenen Ansprüche, das zusätzlich ein Substrat umfasst.

15. Verwendung nach Anspruch 14, worin das Substrat zumindest eines von Polystyrol, Polysulfon, Polymethylmethacrylat und Derivaten beliebiger davon umfasst.

16. Verwendung nach Anspruch 14 oder 15, worin das Substrat eine Faser ist.

17. Verwendung nach Anspruch 16, worin die Faser eine Faser vom Inselstruktur-Typ ist.

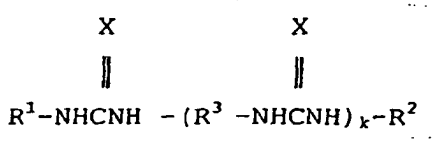
18. Verwendung nach einem der vorangegangenen Ansprüche, worin das Material wasserunlöslich ist.

19. Verwendung eines Materials nach einem der Ansprüche 1 bis 18 zur Beseitigung oder Entgiftung von Superantigenen in vitro.

20. Verwendung nach einem der Ansprüche 1 bis 18, worin das Material in einem Körperflüssigkeits-Reinigungssäule bereitgestellt ist.

21. Wundverbandmaterial, das ein Material nach einem der Ansprüche 1 bis 18 umfasst.

22. Material zur Verwendung bei der Behandlung eines Menschen- oder Tierkörpers zur Beseitigung oder Entgiftung von Superantigenen, **dadurch gekennzeichnet, dass** das Material eine Verbindung der Formel (I'):



(I')

ist, worin

X = O oder S ist;
k = 0 oder eine positive ganze Zahl ist; und
R^1, R^2 und R^3, die gleich oder voneinander verschieden sein können, jeweils ein eine Einheit mit einer zur Bildung einer Wasserstoffbrückenbindung fähigen Gruppe oder eine Einheit mit einem aromatischen Substituenten sind, mit der Maßgabe, dass in der Verbindung eine Einheit mit einer Aminogruppe als zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe und eine Einheit mit einem aromatischen Substituenten enthalten sind.

23. Material nach Anspruch 22, worin die Aminogruppe eine sekundäre oder tertiäre Aminogruppe ist.

24. Material nach Anspruch 22, die auch eine Hydroxylgruppe als zur Bildung einer Wasserstoffbrückenbindung fähige Gruppe enthält.

25. Material nach Anspruch 24, worin die Hydroxylgruppe eine Hydoxylgruppe eines Glucids oder eines Derivats davon ist.

26. Material nach Anspruch 22, worin Einheiten mit einer zur Bildung einer Wasserstoffbrückenbindung fähigen Gruppe und Einheiten mit einem aromatischen Substituenten alternieren.

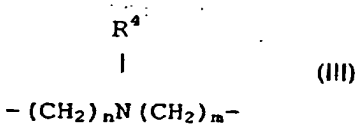
27. Material nach einem der Ansprüche 22 bis 26, worin der aromatische Substituent eine Phenylgruppe, eine Naphthylgruppe oder ein Derivat davon ist, worin zumindest eines der Wasserstoffatome durch F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 oder CH_2PhNH_2 substituiert ist.

28. Material nach einem der Ansprüche 22 bis 27, worin R^1 und/oder R^3 eine Struktur der Formel (II)

(II)

enthält bzw. enthalten, worin n und m jeweils unabhängig voneinander aus 0 und 1-10 ausgewählt sind.

29. Material nach einem der Ansprüche 22 bis 27, worin zumindest einer von R^1, R^2 und R^3 eine Struktur der Formel (III)



(III)

enthält, worin R^4 Wasserstoff oder eine C_{1-10}-Alkylgruppe ist und n und m jeweils unabhängig voneinander aus 0 und 1-10 ausgewählt sind.

30. Material nach einem der Ansprüche 22 bis 29, worin k = 1 bis 200 ist.

31. Material nach Anspruch 22, das eine Grundeinheit aus Polymethylmethacrylat, Polysulfon, Polystyrol oder einem

Derivat beliebiger davon aufweist.

32. Körperflüssigkeits-Reinigungssäule, die ein Material-nach einem der Ansprüche 22 bis 31 umfasst.

33. In-vitro-Verfahren zum Beseitigen eines Superantigens aus einer Flüssigkeit, indem die das Superantigen enthaltende Flüssigkeit durch eine Säule geschickt wird, die mit einem Material zur Beseitigung oder Entgiftung des Superantigens gefüllt ist, **dadurch gekennzeichnet, dass** das Material ein Material nach einem der Ansprüche 1 bis 18 ist.

34. Verfahren nach Anspruch 33, worin die Flüssigkeit Blut, Plasma oder Serum ist.

35. In-vitro-Verfahren zum Beseitigen eines Superantigens aus Blut oder Plasma, umfassend die nachstehenden aufeinander folgenden Schritte:

(a) das Kontaktieren des Bluts oder Plasmas mit einem Material, das zur Beseitigung oder Entgiftung des Superantigens fähig ist, wobei das Material ein Material nach einem der Ansprüche 1 bis 18 ist; und
(b) das Abtrennen des Bluts oder Plasmas vom Material.

Revendications

1. Utilisation, au cours de la préparation d'un agent employé dans le traitement d'un organisme humain ou animal pour l'élimination ou la détoxification de superantigènes, d'une substance contenant une liaison urée ou une liaison thiourée, laquelle substance contient de plus un cycle aromatique et/ou un groupe capable de former une liaison hydrogène.

2. Utilisation selon la revendication 1, dont la substance contient à la fois un cycle aromatique et un groupe qui est capable de former une liaison hydrogène.

3. Utilisation selon la revendication 2, dont la substance est un composé de formule (I)

$$R^1-NHCNH-(R^3-NHCNH)_k-R^2 \qquad (I)$$

lequel dit composé contient un groupe capable de former une liaison hydrogène et un substituant aromatique et,

X est O ou S;
k vaut 0 ou un nombre entier positif; et
chacun de R^1, R^2 et R^3 est l'une quelconque d'une unité ayant un groupe capable de former une liaison hydrogène et d'une unité ayant un substituant aromatique.

4. Utilisation selon la revendication 3, dans laquelle, dans la substance, une unité ayant un groupe capable de former une liaison hydrogène et une unité ayant un substituant aromatique sont répétées en alternance.

5. Utilisation selon l'une quelconque des revendications précédentes dan laquelle un groupe qui est capable de former une liaison hydrogène est un groupe amino.

6. Utilisation selon la revendication 5, dans laquelle le groupe amino est un groupe amino secondaire ou tertiaire.

7. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle un groupe qui est capable de former une liaison hydrogène est un groupe hydroxyle.

8. Utilisation selon la revendication 7, dans laquelle le groupe hydroxyle est un groupe hydroxyle d'un glucide.

9. Utilisation selon la revendication 8, dans laquelle le glucide est au moins un parmi le chitosan, la cellulose et les dérivés du chitosan et de la cellulose.

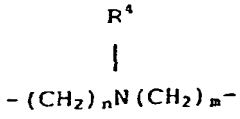
10. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle, dans la substance, le substituant aromatique est un groupe phényle, un groupe naphthyle ou un de leurs dérivés, dont au moins un des atomes d'hydrogène est substitué par F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 ou CH_2PhNH_2.

11. Utilisation selon la revendication 3 ou 4, dans laquelle, dans la substance, R^1 et/ou R^3 contien(nen)t une structure de formule (II)

$$-(CH_2)_nCHOH(CH_2)_m- \qquad (II)$$

dans laquelle n et m sont chacun choisis indépendamment entre 0 et 1 à 10.

12. Utilisation selon la revendication 3 ou 4, dans laquelle, dans la substance, au moins un parmi R^1, R^2 et R^3 contient une structure de formule (III)



(III)

dans laquelle R^4 est un atome d'hydrogène ou un groupe alkyle en C_1 à C_{10} et n et m sont chacun choisis indépendamment entre 0 et 1 à 10.

13. Utilisation selon la revendication 3 ou 4, dans laquelle, dans la substance, k est compris entre 1 et 200.

14. Utilisation selon l'une quelconque des précédentes revendications, d'une substance qui comprend en outre un substrat.

15. Utilisation selon la revendication 14, dans laquelle le substrat comprend au moins un parmi le polystyrène, la polysulfone, le polyméthyl méthacrylate et les dérivés de l'un quelconque de ceux-ci.

16. Utilisation selon la revendication 14 ou 15, dans laquelle le substrat est une fibre.

17. Utilisation selon la revendication 16, dans laquelle la fibre est une fibre du type île dans la mer.

18. Utilisation selon l'une quelconque des précédentes revendications, dans laquelle la substance est insoluble dans l'eau.

19. Utilisation, pour l'élimination ou la détoxification in vitro de superantigènes, d'une substance telle que définie dans l'une quelconque des revendications 1 à 18.

20. Utilisation, selon l'une quelconque des revendications 1 à 18, dans laquelle la substance est fournie dans une colonne de purification de fluide corporel.

21. Substance de pansement de blessure comprenant une substance telle que définie dans l'une quelconque des revendications 1 à 18.

22. Substance pour une utilisation dans le traitement d'un corps humain ou animal pour l'élimination ou la détoxification de superantigènes, **caractérisée en ce que** la substance est un composé de formule (I')

(I')

dans laquelle

X est O ou S;
k vaut 0 ou un nombre entier positif; et
chacun de R^1, R^2 et R^3, qui peuvent être identiques ou différents, est l'une quelconque d'une unité ayant un groupe capable de former une liaison hydrogène et d'une unité ayant un substituant aromatique, pourvu que le composé contienne une unité ayant un groupe amino comme groupe capable de former une liaison hydrogène et une unité ayant un substituant aromatique.

23. Substance selon la revendication 22, dans laquelle le groupe amino est un groupe amino secondaire ou tertiaire.

24. Substance selon la revendication 22, qui contient aussi un groupe hydroxyle comme groupe capable de former une liaison hydrogène.

25. Substance selon la revendication 24, dans laquelle le groupe hydroxyle est un groupe hydroxyle d'un glucide ou d'un de ses dérivés.

26. Substance selon la revendication 22, dans laquelle une unité ayant un groupe capable de former une liaison hydrogène et une unité ayant un substituant aromatique sont répétées en alternance.

27. Substance selon l'une quelconque des revendications 22 à 26, dans laquelle le substituant aromatique est un groupe phényle, un groupe naphthyle ou un de leurs dérivés, dont au moins un des atomes d'hydrogène est substitué par F, Cl, Br, CH_3, C_2H_5, NO_2, OCH_3 ou CH_2PhNH_2.

28. Substance selon l'une quelconque des revendications 22 à 27, dans laquelle R^1 et/ou R^3 contien(nen)t une structure de formule (II)

(II)

dans laquelle n et m sont chacun choisis indépendamment entre 0 et 1 à 10.

29. Substance selon l'une quelconque des revendications 22 à 27, dans laquelle au moins un parmi R^1, R^2 et R^3 contient une structure de formule (III)

(III)

dans laquelle R^4 est un atome d'hydrogène ou un groupe alkyle en C_1 à C_{10} et n et m sont chacun choisis indépendamment entre 0 et 1 à 10.

30. Substance selon l'une quelconque des revendications 22 à 29, dans laquelle k est compris entre 1 et 200.

31. Substance selon la revendication 22 qui a une unité répétée de polyméthylméthacrylate, polysulfone, polystyrène, ou un dérivé de l'une quelconque de celles-ci.

32. Colonne de purification de fluide corporel comprenant une substance telle que définie dans l'une quelconque des revendications 22 à 31.

33. Procédé in vitro pour éliminer un superantigène d'un fluide en faisant passer le fluide contenant le superantigène à travers une colonne remplie avec une substance pour l'élimination ou la détoxification du superantigène, **caractérisée en ce que** la substance est telle que définie dans l'une quelconque des revendications 1 à 18.

34. Procédé selon la revendication 33, dans lequel le fluide est du sang, du plasma ou du sérum.

35. Procédé in vitro d'élimination d'un superantigène du sang ou du plasma, comprenant les étapes successives de

(a) mettre en contact le sang ou le plasma ave une substance capable d'éliminer ou de détoxifier le superantigène, laquelle substance est telle que définie dans l'une quelconque des revendications 1 à 18; et
(b) séparer le sang ou le plasma de la substance.

Fig. 1

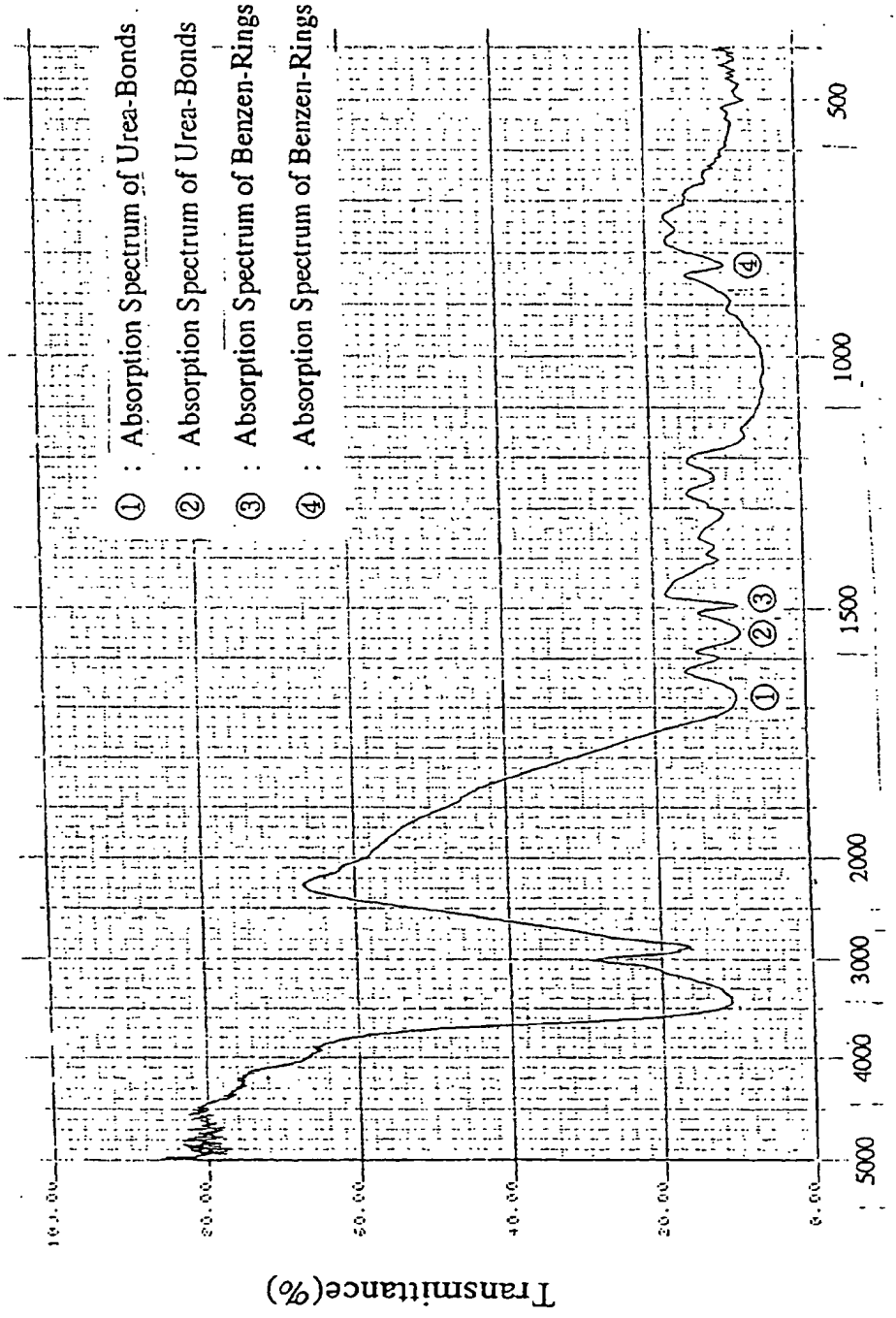

① : Absorption Spectrum of Urea-Bonds
② : Absorption Spectrum of Urea-Bonds
③ : Absorption Spectrum of Benzen-Rings
④ : Absorption Spectrum of Benzen-Rings
Transmittance(%)
Wave Number (cm-1)
5000
4000
3000
2000
1500
1000
500

Fig. 2

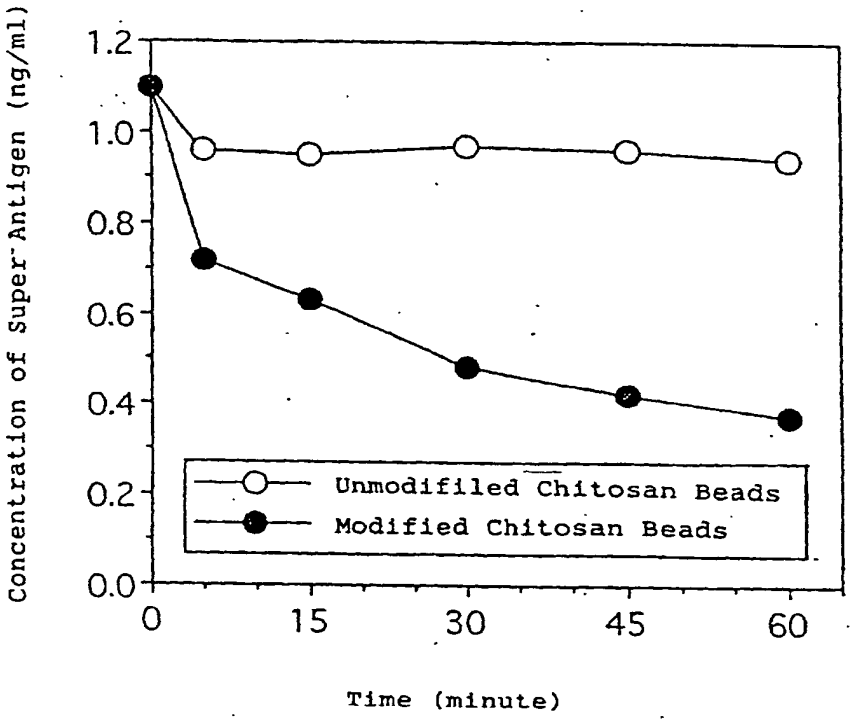
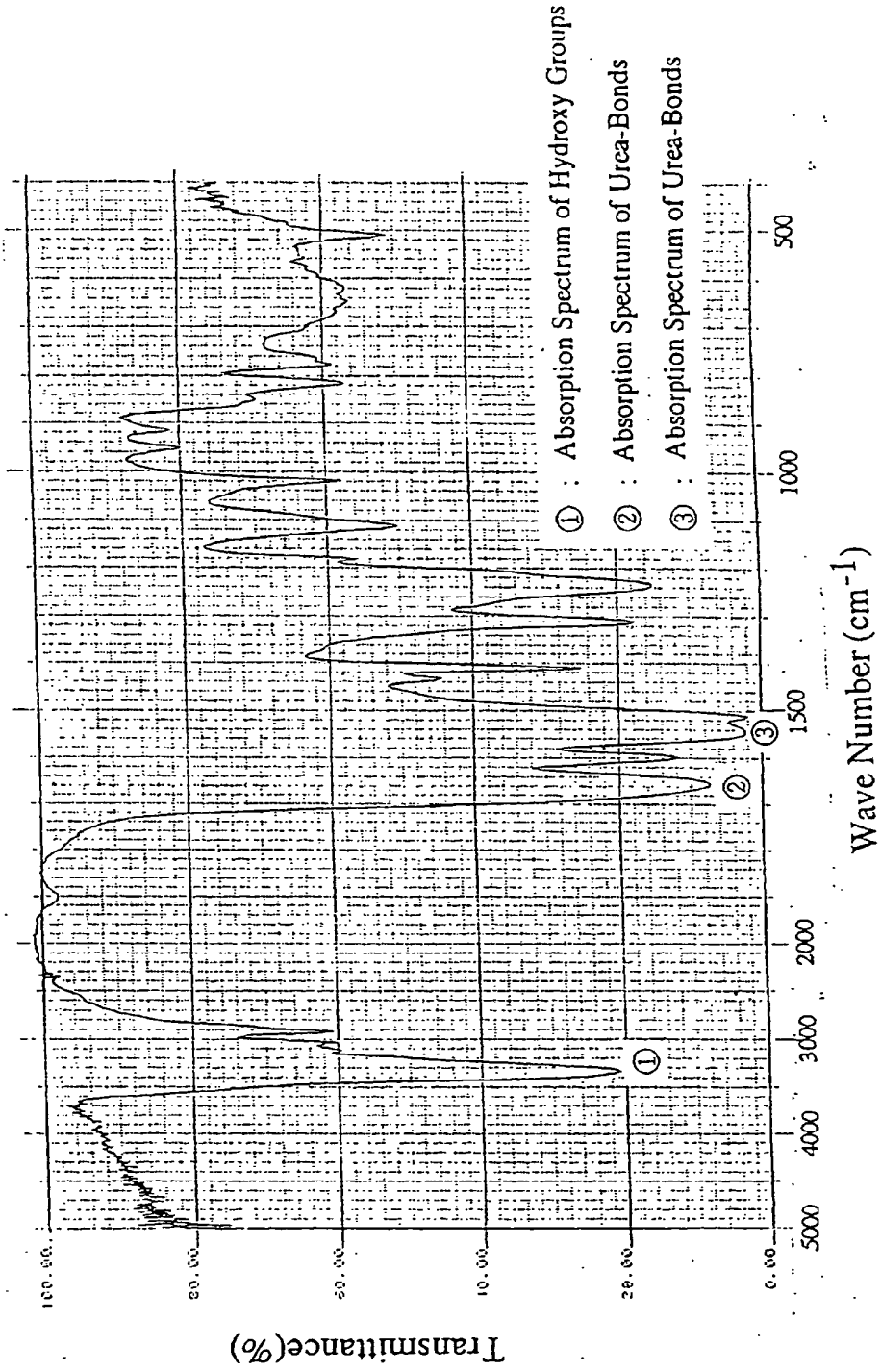

Concentration of Super Antigen (ng/ml)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
15
30
45
60
Time (minute)
Unmodifiled Chitosan Beads
Modified Chitosan Beads

Fig.3


① : Absorption Spectrum of Hydroxy Groups
② : Absorption Spectrum of Urea-Bonds
③ : Absorption Spectrum of Urea-Bonds
Transmittance(%)
Wave Number (cm-1)
100.00
80.00
60.00
40.00
20.00
0.00
5000
4000
3000
2000
1500
1000
500